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RESEARCH ARTICLE

CYTOLOGICAL SPECTRUM OF HEPATIC ASPIRATES: A PROSPECTIVE STUDY AT TERTIARY CARE HOSPITAL OF NORTH INDIA

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ABSTRACT

Background: The ultrasound guided fine needle aspiration cytology has become optimal procedure to establish tissue diagnosis for discrete hepatic masses for their appropriate management.
Aims: To study detailed cytomorphological features of hepatic aspirates and to correlate them with clinical, serum Alpha fetoprotein levels as well with radiological findings in order to distinguish benign from malignant and further to differentiate primary carcinoma from metastases.
Material and Methods: This was a prospective study over period of two and half years,(January 2013 to July 2015) comprising of 150 patients with liver space occupying lesions who underwent ultrasound guided FNA. FNAC was performed under ultrasound guidance using a 21-23 guage lumbar puncture needle, fitted to a 20 ml disposable syringe attached to metallic syringe holder.1-3 passes were made to get adequate aspirates. Direct air dired smears were prepared for May Grunwald Giemsa and few smears were immediately fixed in 95% alcohol for Haematoxylin and Eosin stain. Special cytochemical stains such as Periodic acid Schiff and Ziehl Neilson were done as and when required. The stained FNA smears were examined by two cytopathologists and were categorized as satisfactory and unsatisfactory for evaluation
Results: Out of 150 aspirates 10 aspirates were considered unsatisfactory for evaluation. Out of remaining 140 patients 110 were male and 30 were female. The male to female ratio was 3.6:1. The age of patients ranged from 25 to 80 years and mean age was 55 years. Neoplastic lesions (86.66%) of liver were more common than non neoplastic lesion and further metastatic deposits (66.6%) were more frequent than primary HCC (20%). Serum AFP levels were raised in HCC. HBsAg and HCV antibody positivity was observed in 70% and 6.6% cases of HCC respectively.HCC presented with solitary space occupying lesion in 76.66% patients and as multiple SOL in 33.33% cases. Among the cytomorphological features the presence of predominant trabecular pattern with transgressing vessels (70%), endothelial wrapping (20%), intracytoplasmic bile in tumor cells (33.33%), scattered atypical nuclei (88.33%) and high N/C ratio (86.66%) were the most important features which helped in distinguishing HCC from metastasis.
Conclusion: The present study emphasized on stepwise algorithmic approach to fine needle aspiration diagnosis of hepatic lesions with close coorelation of clinicoradiological and serum AFP with cytomorphological features.

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INTRODUCTION

Hepatocellular carcinoma is the sixth most common malignancy worldwide and the third most frequent cause of cancer death. Hepatocellular carcinoma is the most common primary neoplasm of liver however metastasis to liver is far more frequent than primary liver cancer. About 82% of Hepatocellular carcinoma cases occur in developing countries with high rates of chronic HBV infection. (Parkin *et al*., 2002) Appropriate clinical management of hepatic masses ranges from partial hepatectomy for primary carcinoma to supportive care for advanced metastatic lesion therefore accurate tissue diagnosis of discrete hepatic masses is very important. Ultrasound or computed tomography guided fine needle aspiration cytology has become a popular procedure to establish diagnosis for liver masses. (Chhieng, 2004) Clinical data, and radiological imaging act as an adjuvant in the diagnosis. (Jitendra *et al*., 2013) Alfa fetoprotein is widely used as a serological tumor marker for hepatocellular carcinoma. A serum Alpha fetoprotein level of >500ng/ml is highly

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suggestive of hepatocellular carcinoma but a mild elevation of AFP can be seen in many other liver disorders. (Yap and Peh, 1991) The aim of present study is to evaluate detailed cytomorphological features of hepatic aspirates and coorelating them with clinical, radiological and serum Alpha fetoprotein levels.

MATERIALS AND METHODS

This was prospective study over a period of two and half years (from Janurary 2013 to July 2015) comprising of 150 patients with liver space occupying lesions who were referred to Department of Pathology of our institution for FNAC under ultrasound guidance. Clinical, radiological and details of viral markers as well serum Alpha fetoproteins were recorded. Bleeding time, clotting time and prothrombin time were evaluated in all cases. The FNAC was performed under ultrasound guidance using a 21-23 guage lumbar puncture needle, fitted to a 20 ml disposable syringe attached to metallic syringe holder.1-3 passes were made to get adequate aspirates. Direct air dired smears were prepared for May Grunwald Giemsa and few smears were immediately fixed in 95% alcohol for Haematoxylin and Eosin stain. Special cytochemical stains such as Periodic acid Schiff and Ziehl Neilson were done as and when required. The stained FNA smears were examined by two cytopathologists and were categorized as satisfactory and unsatisfactory for evaluation. Out of 150 patients who under went ultrasound guided FNAC of liver 140 aspirates were satisfactory for evaluation and 10 aspirates were unsatisfactory for evaluation. The stained FNA smears were examined and evaluated for cytomorphological findings and diagnosis. The cytomorphological findings were analyzed and correlated with clinical, and AFP and radiological parameters.

RESULTS

During the present study 150 patients underwent ultrasound guided FNA of liver. The cytological smears were divided into

A) Satisfactory smears: The smears with sufficient cellularity were further categorized as non neoplastic and neoplastic.
B) Unsatisfactory smears: The smears with inadequate material were designated as unstatisfactory for evaluation

Out of 150 aspirates 10 aspirates were considered unsatisfactory for evaluation. Out of remaining140 patients 110 were male and 30 were female. The male to female ratio was 3.6:1. The age of patients ranged from 25 to 80 years and mean age was 55 years. On basis of cytological diagnosis the patients were grouped as non neoplastic and neoplastic with further subcaterorization as shown in Table 1. Non Neoplastic lesions (10) included two cases of pyogenic abscess, two cases were reported as tuberculosis and six cases were reported as reactive hepatocytes. All neoplastic cases were malignant. Out of 130 malignant lesions, majority were metastases (66.6%) and 30 cases (20%) were reported as hepatocellular carcinoma. Among the metastases, metastatic adenocarcinoma was the commonest comprising 80% of cases as tabulated in Table no.4. On ultrasound imaging among non neoplastic group 8(80%) cases had solitary lesion and 2 cases (20%) had multiple space occupying lesions. The size of the lesion ranged from 2cm to 5 cm. In Neoplastic group among HCC category 23 cases (76.66%) had solitary SOL and 7(33.33%) patients had had multiple SOL. The size of lesion ranged from 2.5 to 10 cm. Cirrhosis of liver was radiologically documented in 15/30 patients. The metastatic group showed solitary SOL in 30(30%) patients and had multiple lesions in 70(70%) patients. The size of lesion ranged from 1cm to 8 cm. None of the patients of metastatic group showed cirrhosis. It was observed that 80 % patients diagnosed as HCC had markedly elevated serum AFP (> 500 ng/ml).There was no case of metastatic tumors which showed elevation of AFP levels as shown in Table 2. The serum AFP protein levels were also correlated with degree of differentiation of hepatocellular carcinoma. 27cases (90%) cases of moderately to poorly differentiated HCC showed elevation of AFP levels and 3 cases (10%) of well differentiated HCC had raised AFP.

Table 1. Cytological diagnosis of hepatic aspirates

Diagnosis	**No of Patients**	**% of patients**
Adequate aspirations	140	93.33%
A.Non Neoplastic		
1Pyogenic Abscess	2	1.3%
2.Tuberculosis	2	1.3%
3.Reactive hepatocytes	6	4%
Total	10	6.66%
B.Neoplastic Aspirations		
1.Hepatocellular carcinoma	30	20%
2.Metastatic deposits	100	66.66%
Total	130	86.66%
Inadequate aspirations		
1.Hemorrhagic	6	4%
2.Necrosis	4	2.66%
Total	10	6.66%
Grand total	150	100%

Table 2. Coorelation of cytomorphological diagnosis and serum alpha fetoprotein levels

FNAC Diagnosis	Serum alpha fetoprotein		
	<15ng/ml	>15-500 ng/ml	>500 ng/ml
1.Hepatocellular Carcinoma	0	02	28
2.Metastatic Tumors	100	0	0

Table 3. Cytomorphological features of hepatocellular carcinoma

Features	HCC(30)
1.cellularity	
High	20
Moderate	10
Low	0
2.pattern of arrangment	
Monolayered sheets	0
Predominant trabecular pattern with transgressing vessels	21
Mixed monolayered sheets and trabecular pattern	2
Trabecular pattern and dissociated cells	1
Endothelial wrapping	6
3.cellular details	
A.cell appearance	
polygonal	27
variable	3
B.cell size	
Small	0
Medium	15
Large	15
4nuclear details	
A.bi/mutinucleation	18
B.ansionucleosis	
mild	4
moderate	20
severe	6
C.n/c ratio	
high	26
normal	4
D.nucleoli	
inconspicous	0
visible	10
promient	20
e.intranuclear inclusions	15
f.atypical naked nuclei	25
5.intracytoplasmic bile	10
6.intracytoplasmic eosinoplhilic inclusion bodies	6

Table 4. Metastatic Depoists in accordance with type of differentiation

Type of metastatic depoists	Number of cases	Percentage
1.Metastatic Adenocarcinoma	80	80%
2.Metastatic undifferentiated Carcinoma	10	10%
3.Metastatic Renal Cell Carcinoma	4	4%
4.Metastatic Small Round Cell tumor	1	1%
5. Metastatic Squamous Cell Carcinoma	2	2%
6.Metastatic Malignant Lymphoma	2	2%
7.Metastatic Malignant Melanoma	1	1%
TOTAL	100	100%

Hepatitis B surface antigen was positive in 21 (70%) patients diagnosed as HCC and 2 cases of HCC showed anti HCV antibody positively. Various cytomorphological details of hepatocellular carcinoma were studied as tabulated in Table 3. It was seen that most useful cytological features which favoured diagnosis of HCC included trabecular pattern with transgressing vessels Fig. 1, endothelial wrapping of tumor cells Fig. 2, polygonal shape of cells, atypical naked nuclei, presence of intranuclear inclusions Fig. 3, presence of macronucleoli fig no 4and intracytoplasmic bile. The HCC was classified into well differentiated (5cases) and moderately to poorly differentiated in 25 cases. The metastatic group showed gland formation and multilayered cell clusters in 60% Fig. 5, intracytoplasmic mucin in 10% cases and necrosis in 10%. The table no.4 shows metastatic depoists in liver in accordance with type of differeniation. The final diagnosis was reached by collaborating the cytomorphological details, detailed clinical history with biochemical and radiological parameters.

DISCUSSION

The role of ultrasound guided FNA in diagnosis of hepatic lesions has evolved over the years. Although clinical, biochemical and radiological parameters help to narrow the differential diagnosis but the tissue diagnosis still remains the gold standard. In the present analysis the age group affected was between 30 to 80 years with mean age of presentation was 55 years. This collaborated by the studies done by Ahuja *et al*. (2007) and Talukder *et al*. (2004) who reported that mean age of the presentation of 53 years. A higher incidence was seen in males with male to female ratio of 3.6:1 Alpana Banerjee *et al*. (2012) and Nggada *et al*. (2004) analyzed hepatic aspirates over the period of two years and showed almost similar M:F ratio of 2.3:1 and 2.5:1 respectively.

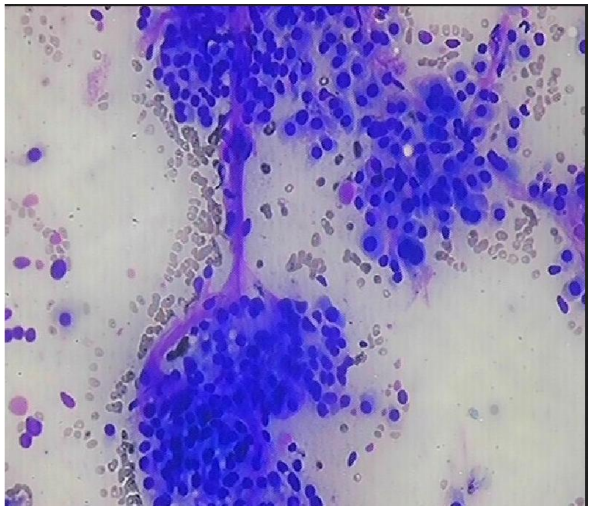

Fig. 1. Cytomorphology showing a transgressing vessel through cluster of hepatocellular carcinoma cells. (Giemsa x40)

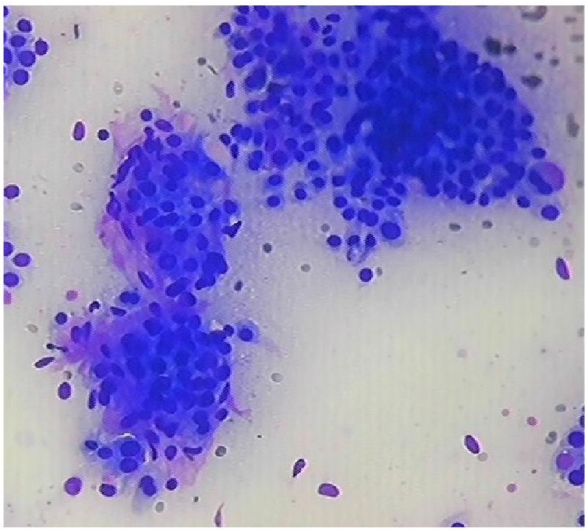

Fig. 2. Endothelial wrapping around cluster tumor cells of hepatocellular cariconoma (Giemsa x40)

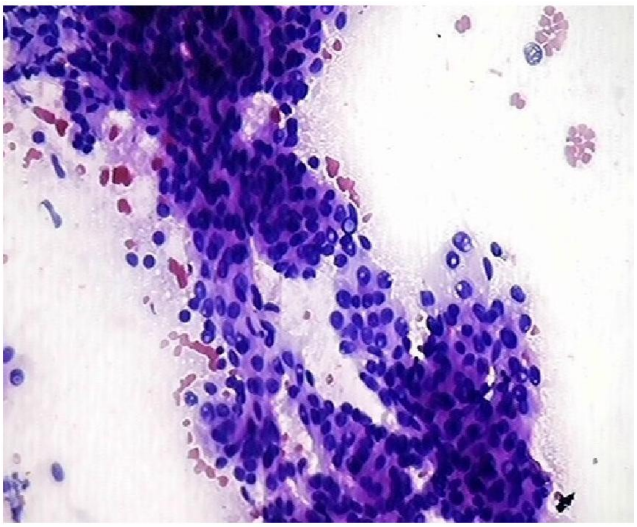

Fig. 3. Intranuclear inclusions in a case of hepatocellular carcinoma (Giemsa x40)

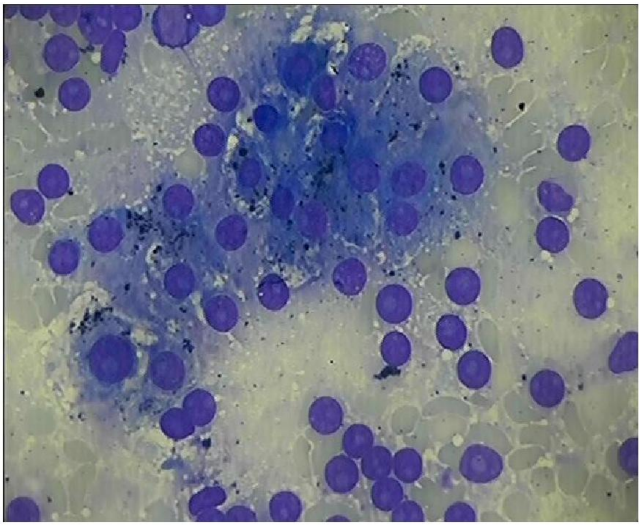

Fig. 4. Macronucleoli in cluster of hepatocellular carcinoma (Giemsa x40)

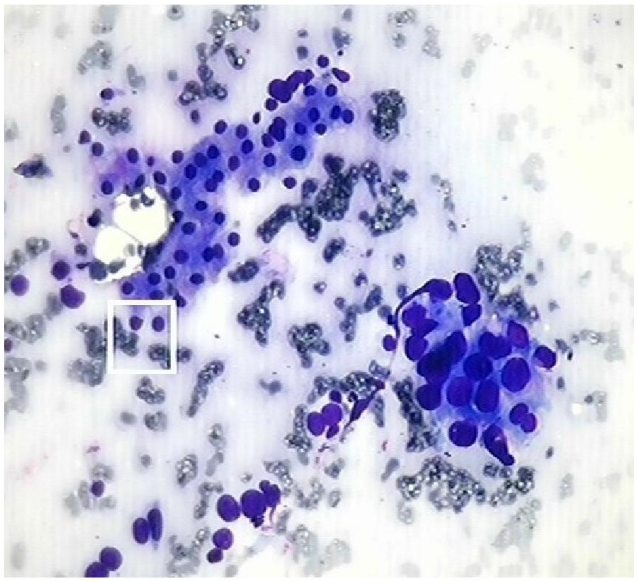

Fig. 5. Clusters of metastatic depoists in liver (Giemsa x40)

The cytological diagnosis in the present study revealed 93.33% smears as adequate for evaluation and remaining 6.6% cases as inadequate for evaluation. A study conducted by Talukder *et al*. (2004) on 108 patients of hepatic aspirates also showed 93.5% aspirates as adequate for evaluation. Similar results were obtained by Rasania *et al*. (2007) who evaluated 90 hepatic aspirates and found 6.67% aspirates as inadequate for evaluation. In current study 140 aspirates were regarded as adequate for evaluation, out of which 10(6.66%) were reported as non neoplastic, 100(66.66%) as metastases and 30(20%) as HCC. Studies done by Talukder *et al*. (2004) reported 73% as metastatic carcinoma and 12% as HCC. Non Neoplastic Lesion: In the current study non neoplastic lesion constituted small proportion (6.66%) of the aspirates. Talukder *et al*. (2004) reported only one case as non neoplastic in their study. Males were affected more (70%) than females. These lesions were commonly seen in the age group of 30-40 years (6 out of 10 cases). Rasania *et al*. (2007) observed that non neoplastic lesions were more common between 20-40 years. In current

study non neoplastic lesions included pyogenic abscess (2), tubercular lesion (2) and reactive hepatocytes in (6). Ziehl Neelson staining was positive in both the tubercular lesion. Alpana Banerjee *et al*. (2012) evaluated 40 liver aspirates out of which two cases were reported as tubercular. Neoplastic lesions:In our study neoplastic lesions were seen in 86.66% cases, similar incidence of neoplastic lesions were reported by Talukder *et al*. (2004) where out of 101 aspirates 100 cases were reported as malignant. HCC was seen in 20% (30) cases and metastases in 66.66% (100) cases during current study. This is in concordance with findings of Jitendra *et al*. (2013) who reported HCC in 27.33% and metastases in 66.67% cases.

Hepatocellular Carcinoma: During current analysis 20% of cases were diagnosed as hepatocellular carcinoma with male to female ratio of 2:1.Talukder *et al*. (2004) also observed that higher incidence of hepatocellular carcinoma in males (7 in 8). HBsAg positivity in cases of HCC in our study were seen in 21patients.Studies conducted by Radhika *et al*. (2004) and Joshi *et al*. (2003) reported similar incidence of 50-70 HBsAg positivity in hepatocellular carcinoma. Hepatitis C virus is another major etiologic factor for HCC in South East Asia. 6.6% of patients of HCC evaluated in current study exhibit HCV antibody positivity. However Ahuja *et al*. (2007) reported a higher prevalence of HCV antibody in cases of HCC (19%) in their study. Alpha fetoprotein estimation have been regarded as a serum tumor marker for HCC. In present analysis 93.33% of HCC cases showed marked elevation (>500ng/ml) and 6.6% of cases showed mild elevation (11-500ng/ml). Our findings are in concordance with previously reported studies from India by Ahuja *et al*. (2007) and Radhika *et al*. (2004) who reported AFP levels elevation in 88.9% and 81.8% of cases respectively. Cirrhosis was radiologically documented in 50% of patients of hepatocellular carcinoma. This is in concordance with study conducted by Ahuja *et al*. (2007) who reported cirrhosis in 40% cases of hepatocellular carcinoma.In current study 76.66% cases of hepatocellular carcinoma presented as single SOL and 33.33% cases presented as multiple SOL. David C. Chhieng (2004) reported hepatocellular carcinoma as single nodule in 82.5% of cases. The cytological features which helped in differentiating HCC from non neoplastic and metastastes have been tabulated in Table 2. It was observed that presence predominant trabecular pattern with transgressing vessel (70%), endothelial wrapping (20%), intracytoplasmic bile in tumors cell (33.33%), scattered atypical naked nuclei (83.33%) and high N/C (86.66%) were the most important features which helped in cytological diagnosis of HCC. Cohen *et al*. (1991) described trabecular pattern in 63 % cases of HCC. Green *et al*. (1984) and Suen *et al*. (1986) reported trabecular pattern as the most common form of arrangement of tumors cells seen in HCC. The predominant trabecular pattern with transgressing vessels was seen (70%) of HCC. This is in concordance with studies conducted by Cohen *et al*. (1991) and Radhika *et al*. (2004) who reported trabecular pattern in HCC in 73% and 63.6% cases respectively. Endothelial wrapping around tumor cells was seen in 20% of cases in our study. Tao *et al*. (1984) observed endothelial wrapping in 50% cases of hepatocellular carcinoma. In present work, we reported intracytoplamic bile in 33.33% cases of HCC. The presence of intracytoplasmic bile was seen in17 to 68 % cases in previously conducted studies from India (Radhika *et al*., 2004; Gupta *et al*., 1986). In current series high N/C ratio in HCC was seen in 86.66% cases. Similar observations were made by Ahuja *et al*. (2007) who reported high N/C ratio in 81.8% of HCC cases. Binucleation and multinucleation was seen in 60% of HCC. Study conducted by Ahuja *et al*. (2007) showed presence of binucleation and multinucleation in 64% of their cases of HCC. Promient nucleoli and multilple nucleoli were observed in 50% and 52% of our HCCcases. Cohen *et al*. (1991) reported slightly higher values with findings of prominent nucleoli in 60% and multiple nucleoli in 54% cases. Atypical naked nuclei were observed in 83.33% of our HCC cases. Studies conducted by Radhika *et al*. (2004), Pedio *et al*. (1988), Cohen *et al*. (1991), revealed presence of atypical naked nuclei in 100%, 93% and 73% cases of HCC respectively. Eosinophilic intracytoplasmic hyaline globules were present in small proportion (20%) of cases of HCC in current study. This is in concordance with findings of Mallikarjuna CM Swamy *et al*. (2011) who reported eosinophilic hyaline globules in 25% of cases of HCC. Cohen *et al*. (1991) emphasized that presence of trabecular pattern of arrangement, atypical nuclei, and High N/C ratio as three primary features for diagnosis for HCC. In current work 100 cases (66.66%) were diagnosed as metastases to liver with majority occurring in age group of 50-60 years. Male to female ratio was 2:1.Most of cases presented with multiple hepatic SOLs. Ahuja *et al*. (2007) reported that mean of patients diagnosed as metastases to liver was 50.28years and male to female ratio of 2.5:1. Metastatic adenocarcinoma was the most common tumor metastasing to liver in our series which is in concordance with findings of Mallikarjuna CM Swamy *et al*. (2011) and Ahuja *et al*. (2007). Cytologically metastatic adenocarcinoma had moderate to high cellularity in 75% of our cases. The pattern of arrangement was predominantly seen as irregular clusters with focal gland formation. The cells were of variable sizes and shapes with high N/C ratio. Intra cytoplasmic mucin was seen in 10% of cases. Similar findings have been reported by Ahuja *et al*. (2007) In the present study no complications were encountered in patients undergoing ultrasound guided FNAC. It was seen that stepwise algorithmic approach which included correlation of cytomorphological features with clinical features, serum AFP levels and radiological parameters aided in accurate diagnosis of hepatic aspirates. The optimal results were obtained with dedicated and expert cytopathologist and radiologist team along with close clinicopathological correlation.

Conclusion

The ultrasound guided FNAC is safe, OPD based, inexpensive procedure which aids in diagnosis of liver lesions and helps to distinguish benign from malignant. This study emphasized on algorithmic approach to FNA diagnosis of hepatic lesions with close clinicoradiological and serum Alpha fetoprotein levels correlation as well as open communication with clinician and radiologists team which is crucial to the success and safety of practice of FNA.

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